EFFECT OF LIGHT AND CARBON DIOXIDE ON THE LOSS OF MANGANESE FROM *CHLORELLA* CELLS[1]

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ABSTRACT

Using radioactive manganese-54, it was shown that appreciably more manganese was lost from *Chlorella* cells in the light than in the dark under conditions which did not permit aeration nor the addition of carbon dioxide. Exposure of *Chlorella* cells to (1) pure nitrogen, (2) 5% CO_2 in nitrogen, (3) air without CO_2, and (4) 5% CO_2 in air indicated that the loss of manganese was due mainly to the absence of CO_2 and not to a deficiency of oxygen.

INTRODUCTION

Manganese is required for autotrophic growth of green plants and for their photosynthesis (Pirson, 1958). Using whole cells of four different algae, *Chlorella pyrenoidosa, Nostoc muscorum, Scenedesmus quadricauda, Porphyridium cruentum,* and whole chloroplasts of *Lemna minor,* it has been shown (Eyster et al., 1956; 1958) that manganese is required in the Hill reaction for the photoreduction of quinone. The effect of various concentrations of manganese in the culture medium on the rates of growth, quinone Hill reaction, and photosynthesis, starting with manganese-deficient cells, was reported.

Preliminary experiments indicated that more manganese was lost from light-exposed than from dark-treated *Chlorella* cells. Because manganese is required for growth, Hill reaction and photosynthesis of green cells, it was deemed important to study more fully some factors which affect the loss of manganese from *Chlorella cells.*

METHODS

Chlorella pyrenoidosa (Emerson strain), obtained from Dr. Jack Myers of the University of Texas, was cultured in Warburg and Burk medium (1950), supplemented with 1 ppm iron as $FeSO_4 \cdot 7H_2O$ made up freshly each week and with the usual A_5 trace element combination, except that manganese, zinc, and copper were added as sulfates instead of as chlorides. The best reagent grade chemicals were used to furnish the macronutrients and Specpure (Johnson, Matthey and Co., Ltd.) provided the iron and trace-element salts ($MnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, H_3BO_3, $ZnSO_4 \cdot 7H_2O$, $NH_4MoO_{24} \cdot 4H_2O$ to furnish 0.5 ppm manganese, 0.02 ppm copper, 0.5 ppm boron, 0.05 ppm zinc, and 0.01 ppm molybdenum, respectively).

Chlorella grown in Warburg and Burk medium containing 0.5 ppm manganese (1×10^{-5}M) were labeled with radioactive Mn^{54}, which is a gamma emitter having a half-life of 310 days. The *Chlorella* cells were grown in 100 ml medium in 300 ml Erlenmeyer flasks, provided with built-in side arms for bubbling with a mixture of 5 per cent carbon dioxide in air. They were illuminated with cool white fluorescent lamps at an intensity of about 800 ft-c. A wrist-action shaker provided continuous agitation of the culture. The 100-ml of medium in the 300-ml flask was labeled with 7 million CPM of radioactive manganese. A pH adjustment to 6.2 was required to neutralize the acidity of the solution of radioactive Mn^{54}. The radioactive medium was inoculated with 1 μl actively growing *Chlorella* cells, which grew in 5 days to a density of 8 μl/ml. The washed cells in each milliliter of medium had a radioactivity of 45,000 CPM.

A study was made to determine the effect of light and dark on the excretion of manganese from the *Chlorella* cells. Eight milliliters of the *Chlorella* culture

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labeled with radioactive Mn^{54} were placed in each of two 12-ml glass centrifuge tubes, which were then centrifuged and the supernatant medium decanted. The cells were washed once by resuspending them in 8 ml of fresh manganese-free culture medium. Centrifuging and decanting were repeated, and then the cells were resuspended in 4-ml of fresh manganese-free culture medium. One tube of *Chlorella* was covered with aluminum foil to prevent exposure to light. Both tubes were placed in a test tube rack in an area receiving 1200 ft-c of cool-white fluorescent light, with an incubation temperature of 25C. At definite time intervals, ½-ml aliquots were removed, to which ½ ml of normal managanese-free Warburg and Burk culture medium was added. These cells were then separated from the culture medium by centrifugation. The culture medium was decanted into planchets, which were allowed to dry at room temperature, after which the Mn^{54} radioactivity was determined on a proportional counter.

For experimental exposures to light in different atmospheres, washed *Chlorella* cells from 1-ml aliquots of the 5-day-old radioactive culture were suspended in 100 ml of manganese-free culture medium in 300-ml sidearmed culture flasks. Those flasks intended for dark treatment were covered with aluminum foil. One pair of flasks was exposed to pure nitrogen. A second pair was exposed to 5 per cent CO_2 in nitrogen, a third pair to air without CO_2, and a fourth pair to 5 per cent CO_2 in air. All of these were supplied by constantly bubbling the gases from compressed sources. The compressed air was bubbled through 0.5 N NaOH for the removal of any trace of CO_2. The exposures were for 24 hours. All the cells in each culture medium were collected by centrifugation, washed twice with distilled water, and then transferred to planchets for radioactivity measurements.

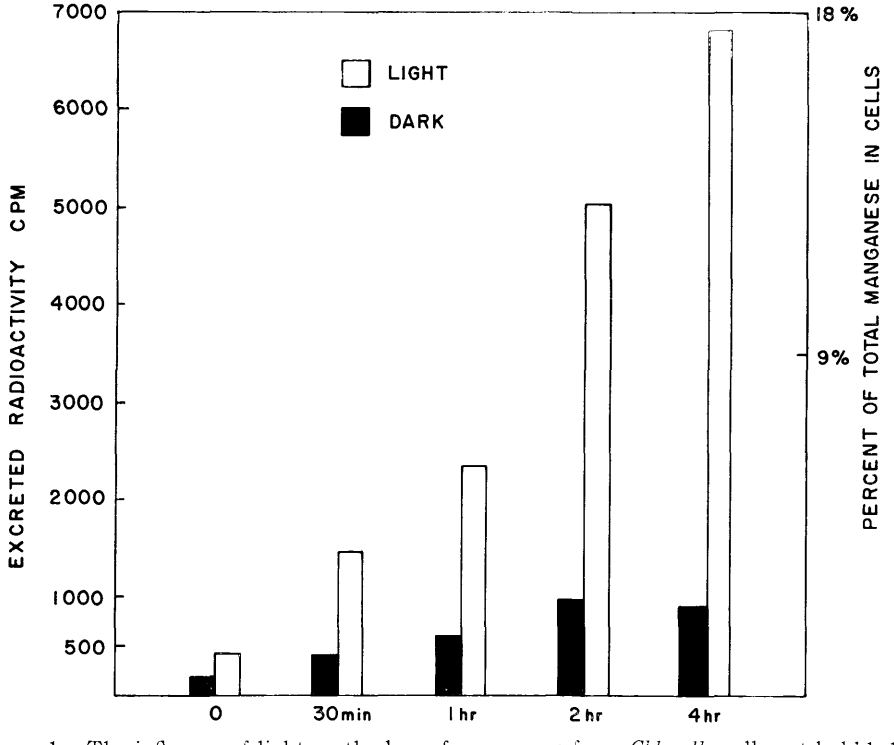

FIGURE 1. The influence of light on the loss of manganese from *Chlorella* cells not bubbled in Mn-free medium, based on the radioactivity of the decanted medium freed of cells by centrifugation.

RESULTS

Figure 1 compares the loss of manganese from *Chlorella* cells exposed to light with similar cells kept in the dark. Appreciably more manganese was lost from *Chlorella* cells after 4 hours in the light (almost 18%) than from those in the dark, where the loss was only about ⅐ as much.

The effect of different gases on the Mn^{54} content of *Chlorella*, in the light and in the dark, is compared in fig. 2. The cells had been cultured and labeled with

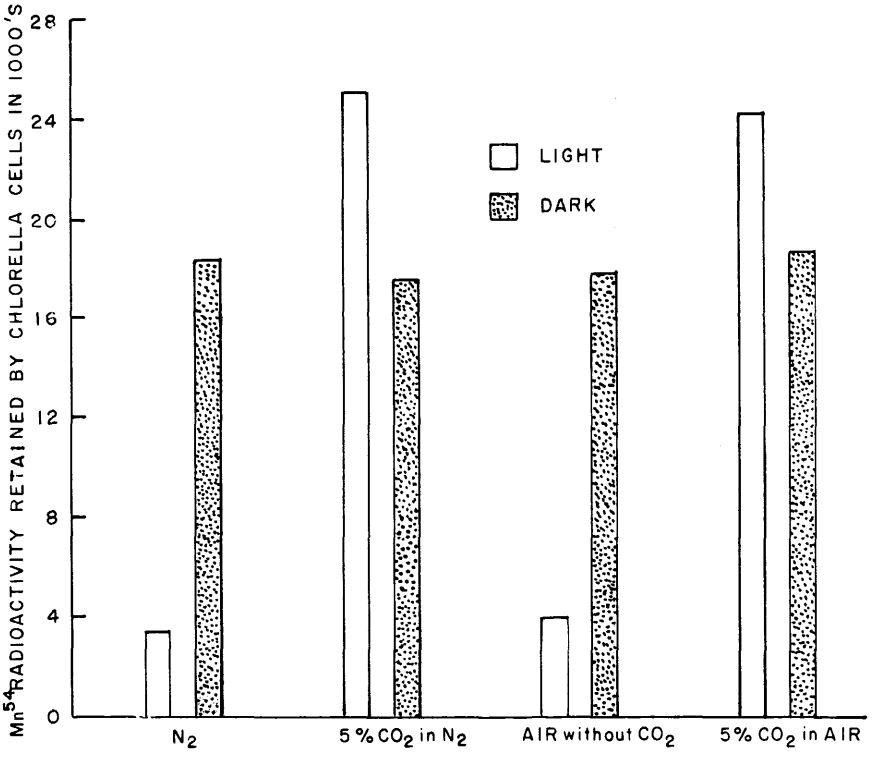

FIGURE 2. The influence of atmospheres of different composition on the loss of manganese from *Chlorella* cells after 24 hrs. exposure in Mn-free medium, based on radioactivity of the cells.

Mn^{54} previously in medium with 0.5 ppm manganese. The results show that, though there was no difference in the amount of Mn^{54} retained by the *Chlorella* cells in the four different types of gases in darkness, in the light there was a great difference. In the presence of CO_2, manganese tended to be retained and the cells grew and multiplied, whereas, in the absence of CO_2, manganese tended to be secreted. Compressed air bubbled through 0.5 N NaOH for the removal of CO_2 caused about as much secretion of manganese as did pure nitrogen. This would indicate that secretion of manganese was due mainly to the absence of CO_2 and not to a deficiency of oxygen.

The rate of manganese secretion from *Chlorella* cells bubbled in the light with N_2 gas was also studied. These results are shown in figure 3. These *Chlorella* cells, labeled with Mn^{54}, had been grown for 5 days in culture medium containing 0.5 ppm manganese and were then placed in manganese-free medium. The loss of manganese was very rapid at first and then slowed down. About 10 per cent

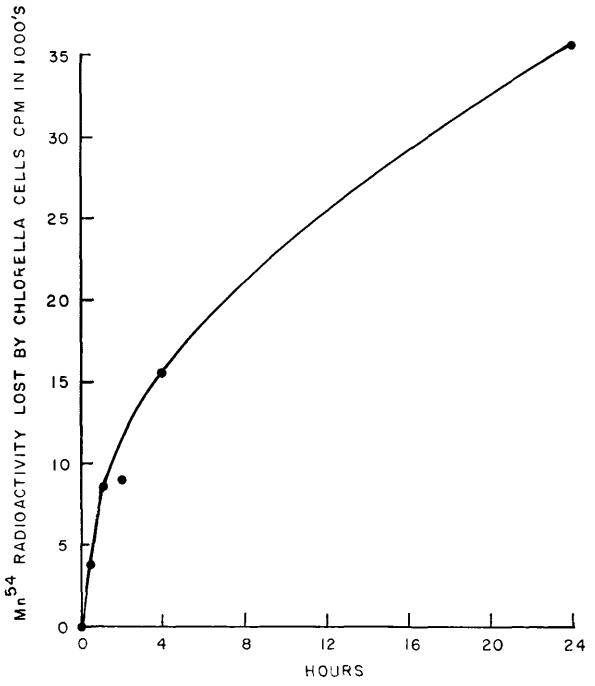

FIGURE 3. The influence of time on the loss of manganese from *Chlorella* cells bubbled in the light with N_2 gas in Mn-free medium, based on radioactivity of the decanted medium freed of cells by centrifugation.

was lost during the first ½ hour, about 20 per cent during the first full hour, and about 80 per cent in 24 hours.

SUMMARY

1. Appreciably more manganese was lost from *Chlorella* cells in the light than in the dark, under conditions which did not provide any treatment with gases.
2. In darkness, there was no difference in the amount of Mn^{54} retained by the *Chlorella* cells when treated with four different types of gases. In the light, there was a great difference, retention being favored by the presence of carbon dioxide in the gas mixture with nitrogen or air.
3. The loss of manganese from *Chlorella* cells bubbled in the light with N_2 gas free of CO_2 was very rapid at first and then slowed down.

LITERATURE CITED

Eyster, C., T. E. Brown, and **H. A. Tanner.** 1956. Manganese requirement with respect to respiration and the Hill reaction in *Chlorella pyrenoidosa.* Arch. Biochem. Biophys. 64: 240–241.

———, ———, ———, and **S. L. Hood.** 1958. Manganese requirement with respect to growth, Hill reaction and photosynthesis. Plant Physiol. 33: 235–241.

Pirson, A. 1958. Manganese and its role in photosynthesis. Chap. 5 in *Trace Elements.* Edited by Lamb, Bentley and Beattie. Academic Press, Inc., New York.

Warburg, O. and **D. Burk.** 1950. Maximum efficiency of photosynthesis. Arch. Biochem. 25: 410–443.